

EXTENDED REPORT

The shared epitope is a marker of severity associated with selection for, but not with response to, infliximab in a large rheumatoid arthritis population

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Objective: To determine whether joint destruction, indication for, and response to infliximab in rheumatoid arthritis are associated with the shared epitope (SE) or selected cytokine gene polymorphisms (interleukin (IL) 1B, IL1-RN, and tumour necrosis α).

Methods: In a large rheumatoid arthritis population of 930 patients from the same area (Rhône-Alpes, France), patients with (n = 198) or without infliximab treatment (n = 732) were compared according to their genetic status. Clinical, biological, and radiological data were collected. Typing for SE status and cytokine polymorphisms was carried out using enzyme linked oligosorbent assay. Statistical analysis was by χ^2 testing and calculation of odds ratios (OR).

Results: A dose relation was observed between the number of SE copies and joint damage in the whole rheumatoid population (OR, 1 v 0 SE copy = 2.38 (95% confidence interval, 1.77 to 3.19), $p < 0.001$; OR 2 v 0 SE copy = 3.92 (2.65 to 5.80), $p < 0.001$). The SE effect increased with disease duration but was not significant before two years. Selection for infliximab treatment (n = 198) was associated with increased disease activity, joint damage, and the presence of the SE with a dose effect. In all, 66.2% patients achieved an ACR20 improvement. No clinical or genetic factors were able to predict the clinical response to infliximab.

Conclusions: This post-marketing study in a large cohort of rheumatoid arthritis patients indicates a linkage between rheumatoid arthritis severity, selection for treatment with infliximab, and the presence and dose of the SE.

Cytokines such as tumour necrosis factor α (TNF α) and interleukin 1 (IL1) are central mediators of joint inflammation and destruction in rheumatoid arthritis.^{1,2} This has been confirmed by the use of cytokine inhibitors.^{3,4} Among the TNF α blockers, infliximab—a chimeric monoclonal antibody against TNF α —is used in combination with methotrexate according to a protocol derived from the ATTRACT study.⁵

Rheumatoid arthritis is associated with a complex genetic component. Many studies have confirmed the association between rheumatoid disease and HLA-DRB1*04.⁶ It has been estimated that approximately one third to one half of the total genetic contribution to rheumatoid arthritis can be attributed to genes in the HLA complex. In two separate genome-wide screens with affected sibling pairs, HLA made the largest genetic contribution.^{7,8} Most of these studies have focused on a direct role for DRB1 alleles that encode a common structural element, designated as the shared epitope (SE).⁹ However, it is unlikely that the SE alone can completely explain the HLA associated risk for rheumatoid arthritis.¹⁰ Furthermore, cytokine polymorphisms have been associated with rheumatoid arthritis susceptibility or severity. In addition to the SE, we focused on different polymorphisms in the IL1B gene (at +3954), in the IL1-RN gene (at +2018), and in the TNFA promoter (at –238 and –308), previously described as severity markers for rheumatoid arthritis.^{11–13}

As TNF antagonists with beneficial effects against joint destruction are available,^{14,15} the identification of genetic and other predictors of rheumatoid arthritis severity and treatment response would provide valuable information for therapeutic decisions. Genetic predictors are particularly

attractive because they can be determined at the time of diagnosis, when therapeutic intervention has the potential to offer the greatest benefit. In the current study, we sought to define the relation between the severity of joint destruction or selection for infliximab treatment and these genetic markers. In addition to the SE, we describe efficacy results of infliximab treatment during this open post-marketing study reflecting the real world, considering these genetic markers as prognosis factors.

METHODS

Patients

From January 2000 to July 2003, 930 consecutive patients who met the American College of Rheumatology (ACR) 1987 criteria for rheumatoid arthritis diagnosis¹⁶ were enrolled in the study. All patients were resident in the Rhône-Alpes area, France, and were followed in four different university hospitals (Edouard Herriot, Lyon Sud, Grenoble, and Saint Etienne). Clinical indices of disease activity and joint destruction included age, sex, disease duration, Ritchie articular index, and right Larsen wrist x ray index. Biological data included: erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and antinuclear antibodies (ANA). Joint damage evaluated by x ray and clinical evaluation was carried out at the same time. Patients were

Abbreviations: ANA, antinuclear antibodies; ATTRACT, anti-TNF trial in rheumatoid arthritis with concomitant therapy; DAS, disease activity score; DMARD, disease modifying antirheumatic drug; EULAR, European League Against Rheumatism; HLA, human leucocyte antigen; SE, shared epitope; SNP, single nucleotide polymorphism; TNF α , tumour necrosis factor α

divided into two groups according to the right Larsen wrist x ray score, as previously described^{11, 17}: destructive arthritis (that is, with a Larsen wrist score ≥ 2), and non-destructive arthritis (Larsen wrist score < 2). Among the 930 rheumatoid patients, 198 received infliximab therapy given at a dose of 3 mg/kg in weeks 0, 2, and 6, and every 8 weeks in accordance to the ATTRACT protocol, in combination with methotrexate.⁵ Rheumatoid patients were selected for infliximab treatment on the basis of persistent rheumatoid arthritis disease activity despite methotrexate treatment.

Each participant gave their written informed consent after receiving information about the study. The protocol was approved by the committee for protection of persons participating in biomedical research.

Outcome measures for patients receiving infliximab treatment

Clinical outcome measures for this study were assessed at baseline and in week 30. Disease activity was assessed according to both the ACR 20 joint response criteria¹⁸ and the response criteria based on the modified disease activity score (DAS) 28 joint index.¹⁹ For this part of the current study, our primary outcome variable was the achievement of an ACR20 response after 30 weeks. The second outcome variables were ACR50, ACR70, and a DAS28 improvement by at least 1.2.

Polymorphism gene typing

Uncoagulated blood was taken from patients and controls and stored frozen at -20°C until DNA extraction. DNA was extracted using the QIAamp DNA blood mini kit (Qiagen GmbH, Hilden, Germany), as recommended by the manufacturer. All genotyping were carried out using an enzyme linked oligosorbent assay (ELOSA) as described previously.²⁰

Shared epitope

Exon 2 regions of both HLA-DR and HLA-B were polymerase chain reaction (PCR) amplified using a combination of DR specific and B specific primers. For the DR specific primer the sequences were: forward primer, 5'CCG GAT CCT TCG TGT CCC CAC AGC ACG-3'; reverse primer, 5'TCG CCG CTG CAC TGT GAA G-3'. For the B specific primer the sequences were: forward primer, 5'GGG AGG AGC GAG GGG ACC G/CC AG-3'; reverse primer, 5'ATC TCG GAC CCG GAG ACT-3'. The amplification mixture was composed of: 50 mM Tris-HCl, pH 8.8, 15 mM ammonium sulphate, 1.5 mM MgCl_2 , 50 μM EDTA, 0.01% (wt/vol) gelatin, 0.2 mM dNTPs, 2.5 U AmpliTaq (Perkin Elmer, Norwalk, Connecticut, USA),

Table 2 Variables contributing to radiological damage and selection for infliximab treatment

	β	95% CI	p Value
Radiological damage			
Age at onset	0.03	1.01 to 1.05	<0.0001
Disease duration	0.14	1.11 to 1.20	0.0002
RF	0.81	1.46 to 3.46	<0.0001
SE positivity	0.69	1.24 to 3.23	0.004
SE homozygosity	0.20	0.46 to 1.46	0.49
Infliximab indication			
Age at onset	0.02	0.96 to 0.99	0.01
Disease duration	0.01	0.99 to 1.05	0.15
RF	0.21	1.80 to 4.13	<0.0001
SE positivity	0.23	0.72 to 1.79	0.59
SE homozygosity	0.26	1.11 to 3.12	0.02

Selected variables were age at onset, disease duration, presence or absence of rheumatoid factor, SE positivity, and SE homozygosity. CI, confidence interval; RF, rheumatoid factor; SE, shared epitope.

0.15 μM for HLA-DR primers, 0.3 for HLA-DR4 primer, and 0.4 μM for HLA-B primers in a 100 μl volume reaction; 50 to 200 ng of extracted DNA were used per amplification. Cycling conditions were as follows: two minutes denaturation at 95°C ; then four cycles of 30 seconds at 95°C , 30 seconds at 68°C , 30 seconds at 72°C ; then four cycles of 30 seconds at 95°C , 30 seconds at 57°C , and 30 seconds at 72°C ; then three cycles of 30 seconds at 95°C , 30 seconds at 64°C , and 30 seconds at 72°C ; then 30 cycles of 30 seconds at 95°C , 30 seconds at 60°C , and 30 seconds at 72°C ; then seven minutes at 72°C . PCR efficiency was checked by agarose gel electrophoresis. Amplicons were hybridised on specific capture probes coated in eight-well strips assembled on a microtitre plate frame, followed by a semiautomated washing, colorimetric detection, and reading. With this method, the SE was defined by the presence of the HLA DRB1*0101, *0102, *0401, *0404, *0405, *0408, *1001, and *1402.

IL1B (+3954)

A single nucleotide polymorphism (SNP) has been described at position +3954 in exon V.^{11, 21} Common allele is C and rare allele is T. Primer sequences and PCR conditions were: forward primer, 5'TTC AGT TCA TAT GGA CCA GA-3'; reverse primer, 5'GTT GTC ATC AGA CTT TGA CC-3'. PCR cycles were: 95°C for two minutes $\times 1$; 94°C for 30 seconds $\times 40$; 55°C for 30 seconds $\times 40$; 68°C for one minute $\times 40$;

Table 1 Odds ratio for articular destruction and selection of rheumatoid arthritis patients for infliximab treatment according to the shared epitope status

	RA without destruction (n = 534)	RA with destruction (n = 396)	OR	χ^2	p Value
SE -/-	267	107	1		
SE +/-	211	201	2.38	33.49	<0.001
SE +/+	56	88	3.92	46.79	<0.001
	RE without infliximab treatment (n = 732)	RA with infliximab treatment (n = 198)	OR	χ^2	p Value
SE -/-	317	57	1		
SE +/-	321	91	1.58	6.01	<0.05
SE +/+	94	50	2.96	24.08	<0.001

Joint damage was 1.7 times more common in patients with two copies of the SE compared with patients with one copy (OR = 1.65 (95% confidence interval, 1.12 to 2.42), $p = 0.01$). Similarly, patients with two copies of the SE were 1.9 times more often selected for infliximab compared with patients with one copy (OR = 1.88 (1.24 to 2.83), $p < 0.01$).

OR, odds ratio; RA, rheumatoid arthritis; SE, shared epitope.

Table 3 Effect of the shared epitope on joint destruction related to disease duration

Disease duration		RA without destruction (n = 534)	RA with destruction (n = 396)	OR	χ^2	p Value
Less than 2 years	SE -/-	22	7	1		
	SE +/-	36	10	0.87	0.06	NS
	SE +/+	8	4	1.57	0.36	NS
Between 2 and 10 years	SE -/-	198	66	1		
	SE +/-	150	112	2.24	18.50	<0.001
	SE +/+	42	42	3.00	18.61	<0.001
More than 10 years	SE -/-	47	34	1		
	SE +/-	25	79	4.36	22.12	<0.001
	SE +/+	6	42	9.67	25.81	<0.001

OR, odds ratio; RA, rheumatoid arthritis; SE, shared epitope.

and 68°C for 10 minutes×1. Amplicons were hybridised on specific capture probes as described for the ELOSA method.

IL1-RN (+2018)

This SNP was described in exon 2 at position +2018.²² Common allele is T and rare allele is C. Primer sequences and PCR conditions were: forward primer, 5'GGG CAC ATG GTG GCT GTG CA-3'; reverse primer, 5'ACC TAG GGT TTG TGC AGG CA-3'. PCR cycles were the same as for IL1B.

TNFA (-238)

This SNP was described in the promoter region of TNFA gene at position -238. Common allele is G and rare allele is A. Primer sequences and PCR conditions were: forward primer, 5'TCA ACG GAC TCA GCT TTC TGA A-3'; reverse primer, 5'CGG AAA ACT TCC TTG GTG GAG-3'. PCR cycles were the same as for IL1B.

TNFA (-308)

This SNP was described in the promoter region of TNFA gene at position -308. Common allele is G and rare allele is A. Primer sequences and PCR conditions were: forward primer, 5'TCA ACG GAC TCA GCT TTC TGA A-3'; reverse primer, 5'CGG AAA ACT TCC TTG GTG GAG-3'. PCR cycles were the same as for IL1B.

Statistical analysis

We used χ^2 tests comparisons involving dichotomous measures and Student's *t* tests for comparisons involving continuous variables. Stepwise methods were used to determine a multivariate model of independent predictors of radiological damage and selection for infliximab

treatment. All analysis were done using StatView software. Probability (p) values less than 0.05 were considered statistically significant.

RESULTS

Dose related effect of the SE on joint destruction

The 930 rheumatoid patients showed the typical clinical and biological features of rheumatoid arthritis. Patients were predominantly women (75%) with a mean disease duration of eight years and a mean number of disease modifying antirheumatic drugs (DMARDs) of 2.3; 64.6% were RF positive and 59.8% carried the SE. SE distribution was 40.2% for 0 copy, 44.3% for 1 copy, and 15.5% for 2 copies. The risk of developing joint destruction was strongly associated with the presence of the SE. SE positive patients were almost three times more likely to develop joint damage compared with the SE negative patients (odds ratio (OR) = 2.70 (95% confidence interval (CI), 2.05 to 3.56), $p < 0.001$). In addition, a dose effect was observed, where patients with one copy of the SE were 2.5 times more likely to have joint damage (OR = 2.38 (1.77 to 3.19), $p < 0.001$ (table 1)). Patients with two copies of the SE were almost four times more likely to have joint damage (OR = 3.92 (2.65 to 5.80), $p < 0.001$ (table 1)).

A stepwise multiple logistic regression analysis was carried out, taking into account variables identified as independent predictors of joint damage. A younger age at onset, a longer disease duration, the presence of RF, and the SE positivity were the most important factors associated with risk of joint damage (table 2). However, this was not significant for the SE homozygosity.

Table 4 Clinical and biological indices of rheumatoid patients according to infliximab treatment

	RA patients without infliximab		RA patients with infliximab		p Value
	Mean (SD) or %	n	Mean (SD) or %	n	
Age (years)	52.7 (14.6)	732	50.4 (12.9)	198	NS
Sex (female)	74.6%	732	78.8%	198	NS
Disease duration (years)	7.5 (7.0)	732	10.4 (8.1)	198	0.01
Ritchie articular index	5.5 (6.8)	576	11.9 (7.3)	198	0.005
Number of DMARDs	2.1 (1.6)	732	3.1 (1.5)	198	0.001
ESR (mm/h)	30.2 (25.6)	666	41.2 (27.6)	198	NS
Rheumatoid factor positive	60.3%	572	79.3%	198	0.01
Antinuclear antibody positive	24.2%	359	22.5%	198	NS
Right Larsen wrist index	1.2 (1.5)	732	2.5 (1.9)	198	0.03

All data were collected at entry and before infliximab treatment.

DMARD, disease modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; SE, shared epitope.

Table 5 ACR response to infliximab according to genetic markers

Marker	ACR ≥ 20 (n)	ACR < 20 (n)	OR	χ^2	p Value
SE –/–	37	20	1		NS
SE +/-	61	30	1.1	0.07	NS
SE +/+	33	17	1	0.01	NS
IL1B (+3954)					
C/C	82	33	1		
C/T and T/T	49	34	0.58	3.24	NS
IL1-RN (+2018)					
T/T	74	34	1		
T/C or C/C	57	33	0.79	0.59	0.06
TNFA (–238)					
G/G	127	62	1		
G/A or A/A	4	5	0.39	1.99	NS
TNFA (–308)					
G/G	99	48	1		
G/A or A/A	32	19	0.82	0.36	NS

ACR, American College of Rheumatology.

Kinetics of joint destruction and the dose of SE

The prevalence of destruction has been shown to increase with rheumatoid arthritis duration. Between 2 and 10 years of disease duration, 36.1% of rheumatoid patients had joint destruction, as defined by a right wrist Larsen index ≥ 2 , versus 66.5% after 10 years. The association of joint destruction with the SE showed a higher odds ratio as disease duration increased, as shown in table 3. Before two years, the SE showed no significant effect on joint destruction. Between two and 10 years, SE positive patients were almost 2.5 times more likely to have joint damage than the SE negative patients (OR = 2.41 (95% CI, 1.70 to 3.40), $p < 0.001$). In addition, a dose effect was observed, where patients with one SE copy were twice as likely to have joint destruction (OR = 2.24 (1.55 to 3.23), $p < 0.001$). Patients with two SE copies were three times more likely to have joint destruction (OR = 3.00 (1.82 to 4.94), $p < 0.001$). After 10 years, the effect of the SE was even stronger. SE positive patients were 5.4 times more likely to have joint damage than the SE negative patients (OR = 5.40 (3.05 to 9.54), $p < 0.001$). In addition, a dose effect was observed. Patients with one SE copy were four times more likely to have joint destruction (OR = 4.36 (2.36 to 8.07), $p < 0.001$) and patients with two SE copies almost 10 times more likely (OR = 9.67 (4.03 to 23.23), $p < 0.001$). Thus the impact of the SE dose on joint destruction increased with rheumatoid disease duration.

Although these results indicate that the association between joint destruction and SE increased with disease duration, this was not related to a change in the patient population, with patients with a benign disease being lost of follow up, as the frequency of the SE remained the same with time (66.7% before 2 years, 56.7% between 2 and 10 years, and 65.2% after 10 years).

Association between the presence of the SE and selection for infliximab treatment

When the two groups were compared, infliximab treated rheumatoid patients had higher disease severity indices (table 4). Patients treated with infliximab ($n = 198$) were slightly younger but this was not statistically significant. Disease duration and Ritchie articular index were significantly higher ($p = 0.01$ and 0.005 , respectively) in this subset. Likewise, they more often had RF and joint destruction ($p = 0.01$ and 0.03 , respectively). ANA frequency was not different.

When comparing the infliximab treated versus the untreated population according to SE status, SE positive patients were almost twice as likely to be selected for infliximab treatment as the SE negative patients (OR = 1.89

(95% CI, 1.35 to 2.65), $p < 0.001$). In addition, a dose effect was observed, patients with one copy were approximately 1.5 times more likely to be selected for infliximab treatment (OR = 1.58 (1.09 to 2.27), $p = 0.01$) and patients with two copies three times more likely (OR = 2.96 (1.92 to 4.56), $p < 0.001$) (table 1).

A stepwise multiple logistic regression analysis was carried out with identified independent predictors of selection for infliximab treatment. As indicated for joint destruction, a younger age at onset, the presence of RF, and the SE homozygosity were the most important factors determining selection for infliximab treatment (table 2). Accordingly, a strong link was observed between selection for treatment with infliximab and joint damage ($\chi^2 = 64.59$, $p < 0.001$).

Effect of the SE and response to infliximab

We next investigated the possible effect of the SE on infliximab response (table 5). The proportions achieving ACR20, ACR50, and ACR70 at 30 weeks were 66.2%, 55.1%, and 31.8%, respectively. The SE had no effect on the degree of response. ACR20 values were 64.9%, 67.0%, and 66.0% in rheumatoid patients with no SE, one SE copy, and two SE copies, respectively. Similar results were observed when responders were defined by the EULAR consensus statement, with 72.2% of the patients being responders.

No effect of the selected SNPs on joint destruction, selection for infliximab treatment, and infliximab response

No link was observed between the selected TNFA (–238 and –308), IL1B (+3954), and IL1-RN (+2018) SNPs and joint destruction or selection for infliximab treatment. In addition, we looked at infliximab response according to these four SNPs (table 5). With respect to the IL1B +3954 genotype, the percentage of patients achieving an ACR20 response was not different enough to reach significance in the C/C group (71.3%) or in the C/T and T/T group (59.0%) ($p = 0.06$). Similarly, no effect was observed with respect to IL1-RN +2018 (68.5% in the C/C group, 63.3% in the C/T and T/T group). Similarly, neither the TNFA –238 nor TNFA –308 genotypes had any effect on the ACR20 response (67.2% v 44.4% (NS), and 67.3% v 62.7% (NS), respectively). Similar results were observed with the DAS28 with respect to these four cytokine SNPs.

DISCUSSION

The association between SE and rheumatoid arthritis susceptibility and severity is well established. However, the gene–dose effect of the SE remains more controversial. Some

studies described this dose effect in populations such as white southern Europeans and Asians, though not in Greeks.²³ In early rheumatoid arthritis, the SE was associated with erosive disease in white subjects.^{24,25} Again in retrospective studies, the presence of a double SE dose was associated with a greater risk of developing rheumatoid arthritis.^{26,27} Other reports did not find such an SE dose effect.²⁸ In this large population, we found a clear gene-dose effect on radiological joint destruction in rheumatoid arthritis. The SE impact increased with disease duration. The same dose effect was observed between the SE and selection for infliximab treatment. These patients with active disease despite methotrexate had both more active and more destructive disease, extending the link between the SE and severity.

To our knowledge, this is the first report showing a dose effect between the SE and selection for anti-TNF α treatment (infliximab, the first commercially available TNF inhibitor). As the effect of the SE on joint destruction was not obvious before the disease had been present for than two years, detection of the SE at an early stage of disease might help to select patients for early aggressive treatment, in particular with TNF inhibitors. Such an early decision might improve the final outcome significantly.²⁹ A potential selection bias was reduced here since during the recruitment period (January 2000 to July 2003) all rheumatoid patients were enrolled in the study in four university hospitals whether or not they were receiving infliximab.

This post-marketing study was conducted to test whether genetic markers could explain the clinically heterogeneous improvement resulting from treatment with infliximab combined with methotrexate. We found no link between the SE status and infliximab response. This may not come as a surprise: as the SE was already associated with selection for treatment one would not expect an additional effect on response. However, previous studies showed that the SE could be a prognosis factor for a DMARD. In fact, SE positive patients were much more likely to achieve a good response if treated with methotrexate-sulfasalazine-hydroxychloroquine than with methotrexate alone. In contrast, SE negative patients did equally well regardless of treatment.³⁰ In a small population of 48 rheumatoid patients treated with etanercept, 55% (six of 11) of the non-responders were SE negative but only 8% of the responders (two of 37).³¹ In another study of 457 rheumatoid patients, two copies of the SE was a significant predictive factor for response to etanercept treatment.³² However, in another small population of 78 rheumatoid patients treated with infliximab, the SE carrier rate was the same in responders and in non-responders.³³ In this study, it was suggested that genetic determinants inside the HLA complex could predict the response to infliximab.

We found no significant linkage between some selected cytokine SNPs and the clinical response to infliximab. Surprisingly, no difference in the ACR20 response was observed in our population with respect to the TNFA -308 genotype. In a previous study involving 59 rheumatoid patients, Mugnier *et al* found that those carrying the rare allele A were twice as likely to have no response as those with the common G/G genotype.³⁴ A higher level of TNF α production in different conditions has been associated with the rare allele A at position -308 of the TNF promoter.³⁵⁻³⁷ The explanation for the discrepancy between our study and Mugnier's is unclear. The two populations were both from France, with a rather similar genetic background, although our study was more powerful, with four times as many patients. The rheumatoid disease activity appeared the same in the two studies (DAS28 = 5.36 (1.08) *v* 5.7 (1.0); NS). However, our data were similar to those showing the absence of an influence of the -308 TNFA SNP on the response to infliximab in Crohn's disease.³⁸

Conclusions

This post-marketing study in a large cohort of patients with rheumatoid arthritis showed a linkage between rheumatoid disease severity and selection for treatment with infliximab. The SE was associated with rheumatoid arthritis severity in the whole population. Furthermore, patients carrying the SE, in particular homozygotes, were more likely to receive such treatment, extending the link to severity. This result needs to be extended to other TNF α blockers. As the effect of the SE on joint destruction only becomes apparent after at least two years of disease evolution, early detection of the SE may be useful for identifying patients at risk of joint destruction and starting protective treatment at an early stage of the disease.

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